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AWARD NUMBER: W81XWH-15-1-0125

TITLE: Potential Application of Viral Empty Capsids for the Treatment of Acute Lung Injury/Acute Respiratory Distress Syndrome

PRINCIPAL INVESTIGATOR: Prof. Ariella Oppenheim

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Jerusalem 91904

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goal of this exploratory project has been to test the hypothesis that recombinant empty capsid of SV40, Virus Like Particles (VLPs), may attenuate ARDS, increasing survival and recovery from this severe clinical condition. The hypothesis was successfully validated. Using the rat 2CLP (cecal ligation and two punctures) model, we found that administration of VLPs reduced mortality from 100% to 25%. The VLPs, or saline control, were administered in 3 equal portions (total dose 0.3 mg/kg VLPs, in saline) for 3 days prior to the 2CLP operation. 2CLP was performed 24 hours later, and animals were sacrificed after additional 24 hours. Body weight of the 2CLP rats of both groups, with and without VLP-administration, decreased sharply after the operation. Two days later VLP-survivors began regaining body weight, at the same rate as the control untreated rats, indicating recovery. The results of the laboratory tests were consistent with body weight data. Tests performed at 24 hours after the operation indicated that all the 2CLP rats, whether they had received saline or VLPs, suffered from severe sepsis. However survivors in the 2CLP+VLPs group showed significantly improved test results when sacrificed 3 days later. Lungs were harvested for pathological examination and for studies on the mechanism elicited by VLPs that attenuate 2CLP-induced sepsis, to be performed as the project continues.					
15. SUBJECT TERMS Acute Respiratory Distress Syndrome (ARDS); Acute Lung Injury (ALI); SV40 virus-like particles (VLPs); sepsis; Cecal ligation and puncture (2CLP); Lipopolysaccharide (LPS).					
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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The proposal is addressed at Acute Respiratory Health Problems, ARDS. It is aimed in particular to develop preventive therapeutics to reduce the incidence of acute respiratory distress syndrome after acute lung injury in trauma patients, by treatment with SV40 Virus Like Particles (VLPs). The rationale is based on the beneficial effect of SV40 VLPs on an Acute Kidney Injury (AKI) model in mice, previously demonstrated by our group (Butin-Israeli, et al, 2008, PloS one 3:e2998. 10.1371/journal.pone.0002998). Both AKI and ARDS are severe clinical conditions that require treatment in intensive care units (ICU). Both are characterized by high morbidity and mortality, in spite of high cost optimal supportive care. All therapeutic treatments attempted so far failed. Previous therapeutic approaches have been single-targeted, aimed at a single target mostly within the immune response. Our novel development is multi-targeted: The VLPs induce complex network when entering cells, including anti-apoptotic signaling which, as was demonstrated, protect mice kidneys from apoptosis, necrosis and consequent damage induced by a toxic (mercury) insult, increasing survival significantly. The intermediary results of the present project shown below demonstrate dramatic effect on survival of rats with sepsis-induced ARDS.

1. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Acute Respiratory Distress Syndrome (ARDS); Acute Lung Injury (ALI); SV40 virus-like particles (VLPs); sepsis; Cecal ligation and puncture (2CLP); Lipopolysaccharide (LPS).

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the USAMRAA Grants Officer whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Efficacy of VLPs in treatment of the LPS-ARDS rat model.

Our experiments showed that the endotoxin model was too mild for our purpose. Therefore after we stopped the studies planned on that model and moved forward to Specific Aim 2. This change was approved by our officer Dr. Usamah Kayyali (letter dated May 11). The change is reflected in the updated timeline shown in the **modified SOW**.

Specific Aim 2: Efficacy of VLPs in treatment of the 2CLP-ARDS rat model

Major Tasks 6,7, including the milestones, were completed on time, by month 10, (modified SOW).

Major Task 8: disease progression and mechanism of VLP protection of the 2CLP model. This major task and its milestones are almost completed, month 13.

Specific Aim 3: Development of a simplified reagent (“lead compound”).

Major Task 9: Construction of plasmids was completed on time, but one of the constructs seems to be faulty and needs repeating.

Major Task 10: Expression and production of the VP1 derivatives. Expression and production of two derivatives was completed on time. The third is delayed as explained above.

Subtask 3, Comparing the proteins for their efficacy in ARDS-arrest in both models and selecting the most effective. The two derivatives whose production was completed underwent preliminary testing for efficacy in ARDS arrest. The third is delayed.

Specific Aim 4: The mechanism of ARDS attenuation by VLP in the 2CLP model

Since the LPS ARDS model was discontinued, we have not yet started to work on Specific Aim 4. The work on this specific aim will begin soon.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1: Efficacy of VLPs in treatment of the LPS-ARDS rat model.

To be tested through assessment of lung pathology, the inflammatory state, oxidative stress and damage to alveolar and endothelial cell in VLP-treated in comparison to untreated ARDS animals.

We realized that the endotoxin model was too mild for our purpose. The insult caused death in only a small proportion of the animals, not allowing us to see whether VLPs had any effect without performing a number of tests, such as cytokines, on a large number of animals to achieve statistical power. Furthermore, there was no correlation between the LPS dose and death rate (Figure 1).

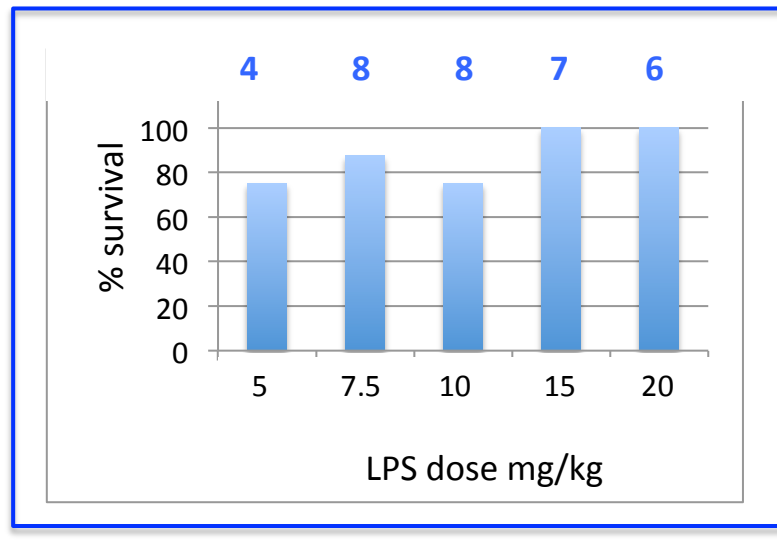


Figure 1. The effect of LPS dose on rat survival.

The number of animals that received each dose is designated above. Note that at the higher LPS doses, 15 and 20 mg/kg, survival was 100%.

We decided to move forward to the other ARDS model that was included in the proposal, the 2CLP model, Specific Aim 2. This change was approved by our officer Dr. Usamah Kayyali (letter dated May 11).

Specific Aim 2: To test the efficacy of VLPs in the treatment of ARDS in the 2CLP model, similarly to Aim 1.

Major Tasks 6 was completed on time, and both milestones, obtaining ACURO approval and establishment of the animal model were achieved earlier than planned, on Month 9th instead of month 10th.

Major Tasks 7 included establishing VLP dose response and delivery time. The **milestones** were achieved on time:

1. Protocol for protective VLP administration for 2CLP induced ARDS
2. Data both on effective dose range and on VLP toxicity
3. Proof of principle for therapeutic effect of VLP for 2CLP induced ARDS

* Milestones

Milestone 1: Protocol for protective VLP administration for 2CLP induced ARDS:

Essentially we found that the protocol that was optimal in our study on the mouse AKI model were also optimal with the 2CLP rat model. VLPs were injected on 3 subsequent days, 1/3 dose on each day, prior to the insult. See full protocol below.

Milestone 2: Data both on effective dose range and on VLP toxicity:

Dose response was tested within the range of 0.1 to 1.0 mg/kg. The optimal dose was found to be 0.3 mg/kg. Because of the high expense in producing large quantities of VLPs we did not go beyond 1.0 mg/kg. Therefore, at this stage we do not know at which dose VLPs become toxic to rats.

Milestone 3: Proof of principle for therapeutic effect of VLP for 2CLP induced ARDS.

The survival graph (Figure 2) shows that 18 rats were sufficient to prove a significant effect of VLPs on survival of 2CLP-operated rats.

Protocol for protective VLP administration for 2CLP induced ARDS (Milestone 1)

Days 1,2,3: Weigh rats.

Tail vein injection of either VLPs (in saline) or saline. VLP concentration was adjusted so that each rat received 100 µl per 100 gram of its weight. Saline was injected at the same times and at the same proportional volume per rat. The daily injection VLP dose is 0.1 mg/kg, a total of 0.3 mg/kg in 3 days.

Day 4: Weigh rats. Perform the 2CLP procedure. Start observing the rats in the late afternoon.

From Day 5: Observe the rats several times during the day. Record death.

Results

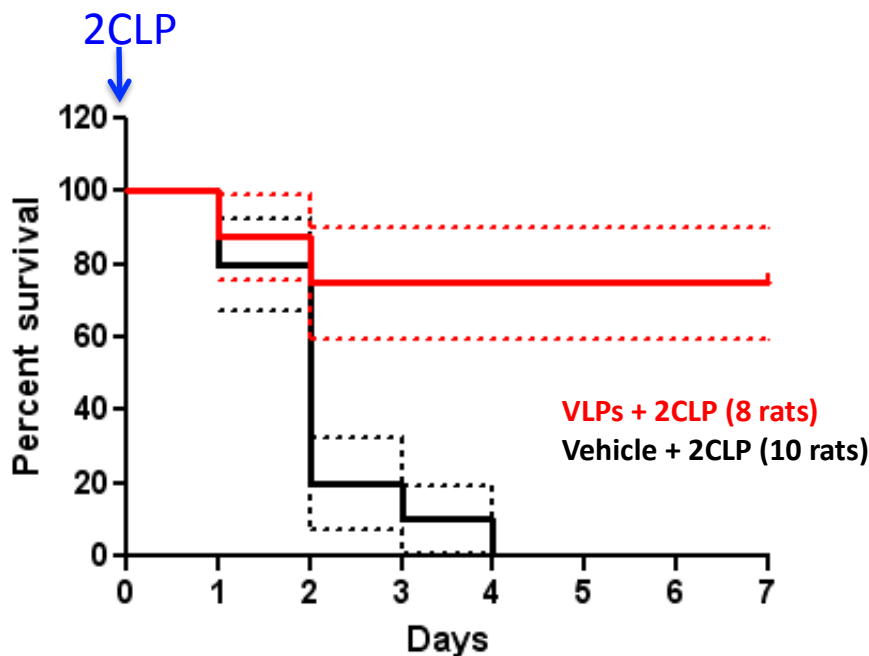


Figure 2. SV40 VLPs significantly increase survival of ARDS-model rats. Rats were injected with a total dose of 0.3 mg/kg VLPs (or saline), divided into 3 equal portions for 3 consecutive days (0.1 mg/kg VLPS/day). All the 18 animals were operated for 2CLP on the 4th day, depicted here as day 0.

Statistical analysis using Log-rank (Mantel-Cox) Test indicated that the results were significant. $P = 0.0026$

Major Task 8: disease progression and arrest

The animals were treated according to the protocol established above for recording survival with the following modification: the 2CLP operation was performed on day 4, as in the protocol. However most of the rats were sacrificed on day 5, 24 hours after the 2CLP operation. Weight was recorded daily. Blood was withdrawn and organs harvested for pathology. One lung of each rat was

frozen in liquid N₂ for extraction of RNA and proteins. In order to study the recovery process, additional 2CLP rats were treated with VLPs and survivors were sacrificed on day 8.

The rats were divided into 5 experimental groups: control-untreated, control-untreated+VLPs, 2CLP-operated+vehicle, 2CLP-operated+VLPs and 2CLP-operated + VLPs harvested 3 days later. Summary of their weights along the experiment is depicted graphically in Fig. 3.

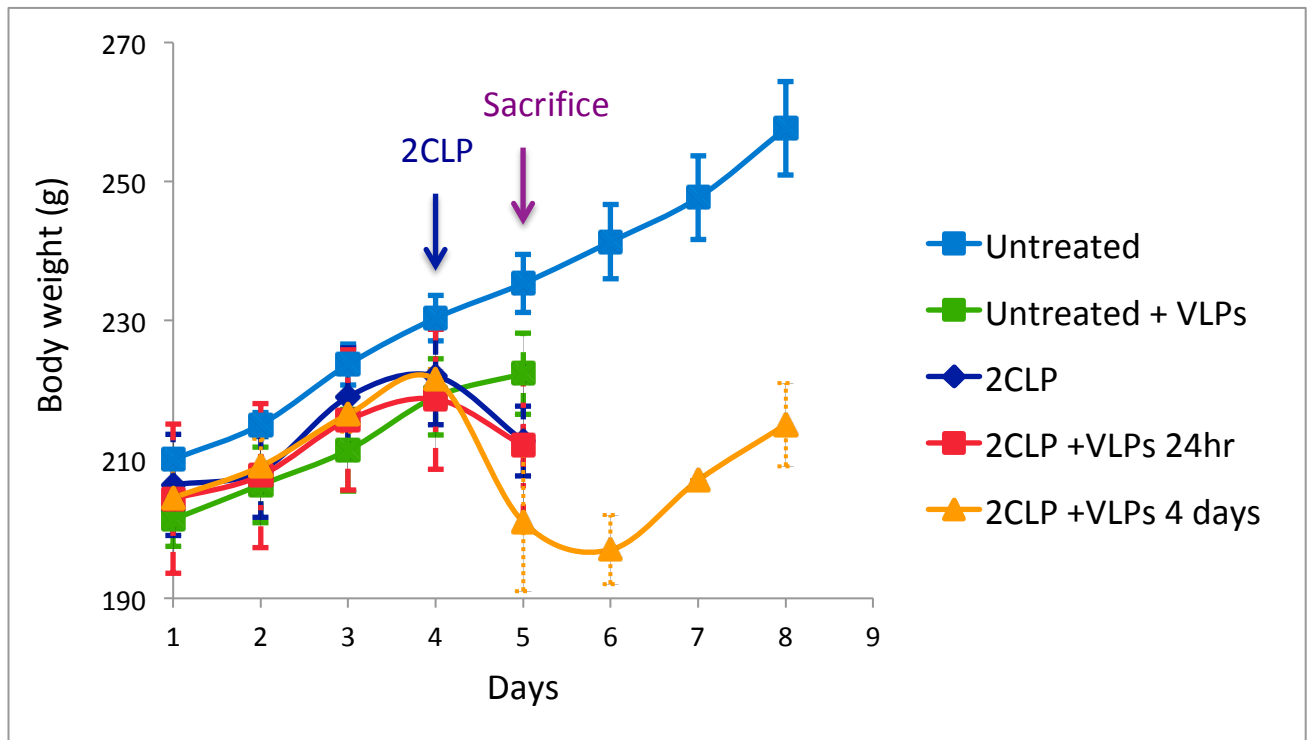


Figure 3. Changes in body weight for the 5 groups of rats. All the rats that underwent the 2CLP procedure on day 4 decreased in their body weight following the operation. Most rats were sacrificed on day 5. The weight of survivors that received VLPs started increasing on day 6, at the same rate as the untreated and the untreated+VLPs, indicating a recovery process.

Preliminary data of blood tests for 5 of the groups are shown in Table 1. In general, the control-untreated and control-untreated+VLP gave similar test results, suggesting the VLPs do not affect the indices tested in healthy animals. The 2CLP-operated were significantly different from the controls in only 3 of the 14 tests performed: Fibrinogen, ALT/GPT and AST/GOT. This may be due, in part, to the low number of rats in each group (3) and the large standard error. It is also possible that rats do not respond to sepsis as humans do. For example, lactate and CRP levels that are usually significantly increased during sepsis in humans, were almost identical in the control and the 2CLP-operated rats (not shown). The same was true for bilirubin and creatinine.

Interestingly, in general the test results of the 2CLP+VLP- harvested 24 hours after the operation were very similar to those of the 2CLP-rats that received vehicle. However 3 days later the values appear to return normal. The data suggest that following the operation rats that receive VLPs suffer a crisis, but the ~75% that overcome the crisis recover.

Table 1. Summary of laboratory tests.

	Laboratory test	Animal Group				
		Untreated	Untreated + VLPs	2CLP Harvest at day 1	2CLP+VLPs Harvest at day 1	2CLP+VLPs Harvest at day 4 (Survivors)
Coagulation	Fibrinogen *	280.5 ± 33.5	224.7 ± 3.7	798.7 ± 7.9	636.0 ± 0.8	336.0 ± 16.6
	PT	24.9 ± 2.9	26.4 ± 0.9	28.4 ± 4.1	31.9 ± 3.6	29.7 ± 2.1
	PTT	19.2 ± 5.1	23.8 ± 0.4	28.7 ± 2.8	28.3 ± 2.8	25.2 ± 3.1
Blood Chemistry	CK	729.0 ± 78.1	433.3 ± 109.3	1934.0 ± 1025.6	721.7 ± 205.2	616.7 ± 112.3
	ALT/GPT *	32.3 ± 3.6	35.1 ± 1.3	78.1 ± 10.8	96.9 ± 9.3	20.1 ± 2.5
	AST/GOT *	95.7 ± 0.8	91.6 ± 6.4	468.3 ± 134.6	389.0 ± 33.2	123.3 ± 29.4
	ALP	866.3 ± 8.3	794.0 ± 68.8	446.7 ± 171.4	413.7 ± 33.8	819.0 ± 132.6
	Urea	33.3 ± 3.6	25.7 ± 0.4	60.3 ± 9.2	101.2 ± 25.9	35.1 ± 5.1
Blood count	WBC	3.9 ± 1.1	2.1 ± 0.2	0.5 ± 0.1	0.3 ± 0.2	6.03 ± 2.5
	Lymphocytes	3.8 ± 1.1	1.7 ± 0.4	0.4 ± 0.1	0.2 ± 0.1	4.0 ± 1.1
	Platelets	687.0 ± 71.9	745.3 ± 66.4	544.0 ± 127.4	551.7 ± 88.4	1323.0 ± 165.3
	Neutrophils	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	2.0 ± 1.4
	RBC	6.9 ± 0.2	6.3 ± 0.1	5.7 ± 0.3	6.2 ± 0.5	5.5 ± 0.2
	HgB	13.5 ± 0.4	12.2 ± 0.1	11.3 ± 0.4	12.3 ± 0.9	10.4 ± 0.4

* A test that showed significant difference between the 2CLP rats and the untreated ones and between the 2CLP rats and the recovered 2CLP+VLP rats. There was no significant difference between the recovered 2CLP+VLP rats and the untreated ones, as expected of full recovery.

The other 2 groups were additional controls for the 2CLP operation, sham-operation control (their abdomen was opened and re-sawn) and sham+VLP control. All the blood tests of these controls did not differ from the parallel 2CLP-operated and the 2CLP-operated+VLPs, and are therefore not shown.

In conclusion, the studies under **Specific Aim 2 provide a proof of principle for the efficacy of VLPs to attenuate ARDS, significantly reducing mortality.** This is seen in the survival study, in their return to normal weight gain and in a number of laboratory tests.

Specific Aim 3. Development of a simplified reagent (“lead compound”).

VLPs cannot be produced in *E. coli*, because the different intra-cellular environment of the bacteria is unfavorable for VLP assembly. We routinely express them in insect cells, using the baculovirus expression system, which is cumbersome and costly. The aim of this part was to develop VP1 derivatives that do not assemble into capsid, and that can be produced in *E. coli*.

We have cloned the VP1 derivatives planned in an expression vector and transformed the bacterial strain BL21. Two of the clones expressed as expected and produced the desired proteins. For these two, Core14 and Core20, we developed a production and purification protocols. They yield 22 and 64 mg/liter culture respectively. For the third we had some problems which we have not finished sorting. Presently we repeat its cloning.

The two VP1-derived proteins, Core14 and Core20, were tested in preliminary experiments for their ability to attenuate ARDS. The preliminary results suggest that Core20 is effective, however at a higher dose than VLPs.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to Report.

How were the results disseminated to communities of interest?

The results obtained so far have been presented soon, as oral presentations, in two Scientific Conferences:

1. **The DNA Tumor Virus Meeting**, Montreal (Quebec) Canada, July 18 -23, 2016
2. **Virus Structure and Assembly**, FASEB Science Research Conference, July 24-29, 2016 Steamboat Springs, Colorado.

Abstracts are attached as Appendix 1

What do you plan to do during the next reporting period to accomplish the goals?

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Specific Aim 2: Efficacy of VLPs in treatment of the 2CLP-ARDS rat model.

The only unfinished task of this specific aim is part of **Major Task 8: disease progression and arrest**: a blind pathological examination of lung specimens harvested from the rats whose data is presented in Fig. 3 and in Table 1.

Specific Aim 3: Development of a simplified reagent (“lead compound”).

We will test 3 lead-compounds, derivatives of VP1 produced in E. coli. The 3 compounds will be compared for efficacy at a range of doses.

Specific Aim 4: The mechanism of ARDS attenuation by VLP in the 2CLP model. This part will be intensively studied during the next 6 months of the project, as described in the original proposal.

4. IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

Proof of concept of the efficacy of SV40 VLPs in attenuating ARDS, preventing death and facilitating recovery of a significant proportion of the 2CLP model ARDS rats.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

We originally planned to also use a toxic ARDS model, induced by E. coli liposaccharide (LPS). However we found that the disease induced by LPS was too mild for testing for the

effect of VLPs. While all the rats showed signs of illness around 24 hours, they almost all recovered afterwards. We therefore stopped those efforts and proceeded directly with the 2CLP model.

The change was approved by our officer Dr. Usamah S Kayyali, in his letter of May 18, 2016.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. Examples of products include:*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award. There is no restriction on the number. However, agencies are interested in only those publications that most reflect the work under this award in the following categories:

Oral Presentations at scientific meeting:

1. **The DNA Tumor Virus Meeting**, Montreal (Quebec) Canada, July 18 -23, 2016.

2. **Virus Structure and Assembly**, FASEB Science Research Conference, July 24-29, 2016 Steamboat Springs, Colorado.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Provide the following information on participants:

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Participants

Name:	Prof. Ariella Oppenheim
Project Role:	PI
Researcher Identifier:	None
Nearest person month worked:	4
Contribution to Project:	Directed the project
Funding Support:	This project.

Name:	Dr. Rohit Srivastava
Project Role:	Postdoc Researcher
Researcher Identifier:	None
Nearest person month worked:	9.5
Contribution to Project:	Performs and directs the animal experimentations. Assists in summarizing data.
Funding Support:	This project and BSF grant 2013041

Name:	Dr. Shashi Gandhi
Project Role:	Postdoc Researcher
Researcher Identifier:	None
Nearest person month worked:	10
Contribution to Project:	Production and purification of VLPs. Cloning, production and purification of VP1 derivatives. Assists in animal experimentations and summarizing data.
Funding Support:	This project and BSF grant 2013041

Name: Mrs. Carol Levi
Project Role: Laboratory Technician
Researcher Identifier: None
Nearest person month worked: 6
Contribution to Project: Assisting in animal experimentations.
Funding Support: This project

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS:

Quad Chart, Attached as Appendix 2.

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Appendix 1: Abstracts of oral presentation at two Scientific Conferences during July 2016.

Appendix 2: Quad Chart.

Appendix 1.

I. Oral Presentation at:

The DNA Tumor Virus Meeting, Montreal (Quebec) Canada, July 18 -23, 2016

Signaling pathways elicited by SV40 harnessed for therapy of critical clinical conditions.

Rohit Srivastava¹, Shashi Gandhi¹, Carol Levi¹, Orly Ben-nun-Shaul¹, Arie Eden² and Ariella Oppenheim¹

¹Hebrew University-Hadassah Medical School, Jerusalem, and ²Carmel Medical Center, Haifa, Israel.

Our research on cellular signaling elicited by SV40 showed that immediately following adsorption the virus induces a complex, robustly balanced network. The network comprises host response to the viral attack and virus induced signaling that overcomes host response. It includes Ca⁺⁺ signaling, caspases, stress response (chaperones), Akt-1 survival pathway and anti-apoptotic signals. These signals are required for SV40 cell entry during early steps, prior to its nuclear entry and viral T-antigen expression, before T-antigen takes over control of viral DNA synthesis. Not surprisingly, the same (or similar) signaling pathways are induced by SV40 VLPs, composed exclusively of recombinant VP1, without any genetic material.

Using a mouse model for toxic Acute Kidney Injury (AKI), we demonstrated that systemic administration of SV40 VLPs significantly increased survival from 12% to >60%. We further showed that AKI attenuation was achieved by upregulation of Hsp70 and activation of Akt-1 survival pathway. Presently we investigate whether SV40 VLPs might also ameliorate sepsis.

Sepsis syndrome is characterized by multiple organ injury due to uncontrolled inflammatory response and leads to significant morbidity and mortality even with current medical advances. The treatment of sepsis is limited to control the infection source, antibiotics, and supportive therapy for failing organs. Over the past few decades multiple attempts have been made to control the immunological response to sepsis. However, while successful in small animal studies, these interventions failed in clinical trials. We reasoned that the failure was because the interventions were aimed at single targets within the immune response. Our approach, on the other hand, is to target multiple endogenous signaling pathways, shaped by evolutionary forces of virus-host interactions

Using a rat model for sepsis, we have recently found that systemic delivery of VLPs dramatically increased survival, from nil to 75%. The mechanisms underlying sepsis attenuation by VLPs is currently under investigation. The implications of this study to the development of a therapeutic mode to treat sepsis and other critical clinical conditions will be discussed.

Supported by the US Army, contract No. W81XWH-15-1-0125

II. Oral Presentation at:

Virus Structure and Assembly, FASEB Science Research Conference, July 24-29, 2016
Steamboat Springs, Colorado.

Signaling pathways elicited by SV40 harnessed for therapy of critical clinical conditions.

Rohit Srivastava¹, Shashi Gandhi¹, Carol Levi¹, Orly Ben-nun-Shaul¹, Arie Eden² and Ariella Oppenheim¹

¹Hebrew University-Hadassah Medical School, Jerusalem, and ²Carmel Medical Center, Haifa, Israel.

Our research on cellular signaling elicited by SV40 showed that immediately following adsorption the virus induces a complex, robustly balanced network. The network comprises host response to the viral attack and virus induced signaling that overcomes host response. It includes Ca⁺⁺ signaling, caspases, stress response (chaperones), Akt-1 survival pathway and anti-apoptotic signals. These signals are required for SV40 cell entry during early steps, prior to its nuclear entry and viral T-antigen expression, before T-antigen takes over control of viral DNA synthesis. Not surprisingly, the same (or similar) signaling pathways are induced by SV40 VLPs, composed exclusively of recombinant VP1, without any genetic material.

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Supported by the US Army, contract No. W81XWH-15-1-0125

PR140862: Potential application of viral empty capsids for the treatment of Acute Lung Injury/ Acute Respiratory Distress Syndrome

PI: Prof. Ariella Oppenheim, Hebrew University of Jerusalem

Budget: \$ 197,224.00

Topic Area: Respiratory Health

Mechanism: Discovery Award

Research Area(s):

Award Status: Open; POP: 01-JUL-2015 TO 31-DEC-2016

Study Goals:

The overall goal is to test the hypothesis that SV40 VLPs are capable of ameliorating ARDS. The hypothesis was to be tested in the two pre-clinical rat models. The study also aimed to provide information with regard to dose, kinetics and pharmacokinetics of the VLPs in this proposed medical application.

Specific Aims:

1. To test the efficacy of VLPs in the treatment of ARDS in the endotoxin (LPS) model through assessment of lung pathology, the inflammatory state, oxidative stress and damage to alveolar and endothelial cell in VLP treated in comparison to untreated ARDS rats.
2. To test the efficacy of VLPs in the treatment of ARDS in the 2CLP model, similarly to Aim 1.
3. To begin developing a simplified reagent ("lead compound").
4. To obtain an insight into the mechanism of VLP attenuation of ARDS, by investigating signaling pathways elicited by VLPs.

Key Accomplishments:

Specific aim 1: Studies on the endotoxin (LPS) model were discontinued because we were not able to achieve 50-80% death in 48 hrs.

Studies on this model were discontinued. VLPs stocks for animal experiments were prepared.

Specific aim 2: Establishing the 2CLP model, which leads to 75% death in 48 hours and additional 20% death in 96 hours.

We found that the effective dose range of VLPs is 0.3 mg/kg, given in 3 equal aliquots at 24 hrs intervals before the insult.

We are approaching the end of collection of clinical data on 6 groups of blindly selected animals. We have also been collecting lung samples, fixed for studies on lung pathology and frozen for RNA and protein studies.

Specific aim 3: Three different plasmids were designed and constructed. Two produce the desired protein were produced and purified. Preliminary experiments suggest that they are effective in counteracting ARDS. The third plasmid is being re-cloned.

Specific aim 4: We are presently starting experiments directed towards investigation of the mechanism of VLP attenuation of ARDS.

Key Outcomes:

1. Animal model for 2CLP-induced ARDS.
2. Protocol for protective VLP administration for 2CLP induced ARDS.
3. Data on the effective dose range and on VLP toxicity.
4. **Proof of principle for therapeutic effect of VLP for 2CLP induced ARDS.**
5. Studies on clinical progress of the disease and its arrest by VLPS through a wide range of laboratory tests on 7 groups of rats.
6. Fixed lungs for pathological examinations.
7. Frozen tissue and slides for studies on disease progress and the protective mechanism through RNA/protein analysis.
8. Two bacterial clones for expression of three VP1 derivatives appear to be effective against ARDS .